Water balance in phasmids and other insects.

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The ability to regulate their water levels very effectively has possibly been one of the most important driving forces in bringing about the huge diversity and number of insect species present in terrestrial environments today. Indeed, insects just in the Order Phasmatodea live in a huge variety of environments, from *Dares* spp. in the rain forests of Borneo to *Gratidia* spp. living in the scorching grasslands of Africa.

Many species of stick insect do drink very readily, for example *Eurycantha* spp., but this is by no means always the case. Spraying of some species in culture can quickly lead to their demise and according to some keepers is entirely unnecessary, if the humidity is at the correct level many of the commoner species will live for many generations without ever drinking. So how do these insects prevent themselves from drying out?

Firstly, it must be noted that many phasmids are not nearly so sensitive to water loss as we are. A human being can lose around 8% of its body weight in water before it becomes lethally dehydrated and dies. Even a camel can only tolerate a 25% decrease in water levels. Most dry-adapted insects, however, can happily survive the loss of nearly 60% of their body water without suffering any harm at all. Naturally, some insects will be more sensitive than others to water loss, but clearly they are in a completely different league from us in this respect.

Even though they may be much more tolerant to water loss than we are, this does not mean that stick insects simply allow water to be lost from them freely. At every point where water might be lost, it seems that the insects as a whole have devised ingenious methods for reducing that loss. In general, insects lose water by the same three routes through which we ourselves lose water. These are: (1) Over the body surface (ie. in insects over the cuticle), (2) From their respiratory surfaces, (3) Via excretion of waste products. Adult female insects also lose water in egg production, but this is largely unavoidable as it is vital that the developing embryo is surrounded by a solution containing the correct concentration of salts and nutrients. The remaining three routes for water loss must all be tightly controlled for the system to work efficiently and effectively in retaining water inside the insect.

(1) The Cuticle

This consists largely of chitin microfibrils, surrounded by a protein matrix. The water content of this varies widely and it is certainly not impermeable to water. This permeability could cause great problems for a stick insect. An important factor for water conservation in any animal is its Surface Area: Volume ratio (SA:V). As any animal gets smaller this gradually increases (there is no need to go into the maths here). Stick insects are relatively large by insect standards, but even so, their SA:V is much higher than our own. This is exacerbated by the fact that a long thin stick insect-shaped cylinder has a much larger surface area than a short fat cylinder of the same volume. Hence, the SA:V will be even higher for a phasmid. Also, leaves are specifically shaped to give maximal SA:V by being thin and flat, just the same shape as a leaf insect. With more surface area, there is more chance for water loss over the cuticle. How then is this prevented?

On the surface of the cuticle is the cuticulin layer, a mixture of waxes and lipids just one molecule thick that lies just over the last proteinaceous layer of the exocuticle. This may be protected by a cement layer which can help prevent mechanical damage to the wax. The cuticulin layer is very important, as this layer is almost entirely responsible for preventing water loss over the cuticle, as can be demonstrated if you dust an insect with alumina powder. The powder, due to its gentle abrasive properties, rubs off the wax layer and consequently the insect will lose water as rapidly as an earthworm in the same ambient conditions. This treatment has no long term effects on the insect, as it has wax channels within its cuticle from which it can renew this wax layer either when it is damaged or when it is stretched as the cuticle expands during feeding.

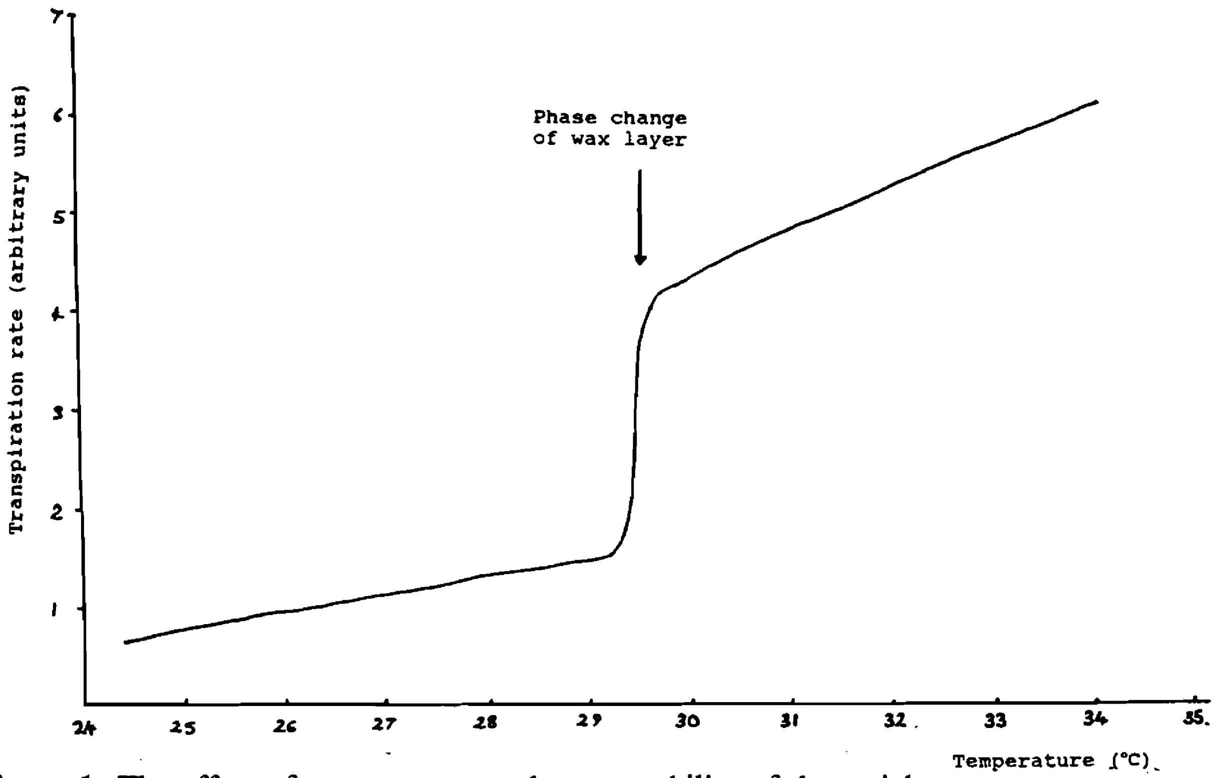

Figure 1 The effect of temperature on the permeability of the cuticle.

The wax is thought to be in a semi-crystalline state; if you heat up an insect, at 29°C there is suddenly a large jump in transpiration (ie: water loss) rate from the insect; see figure 1. The temperature at which this phase transition takes place obviously will vary between species, but possibly sets the upper temperature at which the insect can effectively control its water content without having to imbibe large quantities of water. The wax layer is situated near the surface of the insect because, during ecdysis, the insect will try to minimise its losses by reabsorbing as much of its old cuticle as it can. An impermeable lipid layer would prevent this reabsorption and so must be kept to the outermost layers of the cuticle.

(2) Respiration

When an organism is respiring, it faces a conflict of interests in a dry environment. Oxygen will only cross a cell membrane in solution. This means that any respiratory surface must be kept moist. However, this leaves the insect with a large surface area from which water may readily evaporate, dehydrating the animal very quickly.

Phasmids, like most other terrestrial insects, respire via a tracheal system: a network of internal tubes which carry air directly to the respiring tissues. This internalisation of the respiratory surfaces is common to most terrestrial animals, but insects really excel in reducing water loss from their respiratory surface by maintaining tight control over their spiracles. Spiracles are the small valves on the surface of the thorax and abdomen of the insect, one pair for each obvious segment in stick insects. They resemble eyes (see diagrams of an abdominal and thoracic spiracle: figures 4 and 5) and the "eyelids" operate under precise nervous control to change the aperture of the opening. This process allows what is known as "cyclic respiration". If you measure the carbon dioxide (CO_2) output and oxygen (O_2) intake of an insect (no easy task, as they respire very slowly), you can see that, in dry conditions, the two do not appear to be linked in any way (see Figure 2). How is this pattern of ventilation achieved through just a single opening?

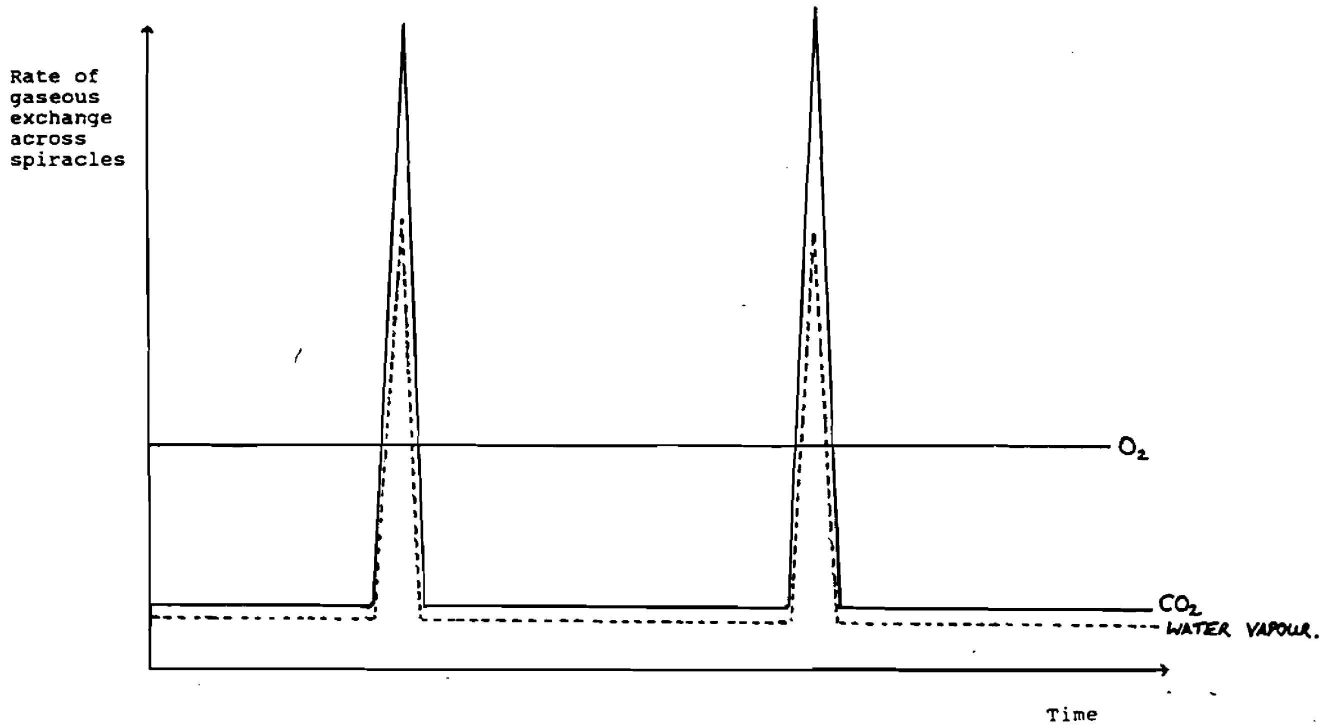

Figure 2 Cyclic respiration.

The process is very elegant and occurs in two stages:

i) The spiracle is almost closed as in figure 3(i), allowing only a very small entry channel for the air. O_2 is used up by the cells, and CO_2 produced. However, CO_2 is much more soluble in water than O_2 and therefore tends to remain in solution instead of crossing the cell membrane into the tracheole. This causes a reduction of pressure in the trachea, which, just like sucking on a straw, draws air into the open end (especially O_2 as it also is diffusing down quite a steep concentration gradient). The miniature "wind" created in this way effectively blows any water vapour away from the spiracle, so almost completely blocking water loss.

ii) Of course, this means that CO_2 levels will be building up in the cells and tracheae and so this potentially harmful waste product will eventually need to be expelled. This is done by opening the spiracles wide and actively pumping the CO_2 out of the cytoplasm in the cells as shown in figure 3(ii). This also allows water to leave the spiracles quite easily, but this phase is only short-lived, and the water curve follows roughly the CO_2 curve on the graph, so that in the long term, the water loss is much less than if the spiracles were open all the time.

If you have a magnifying glass (and a fair amount of patience!) you can watch the spiracles on

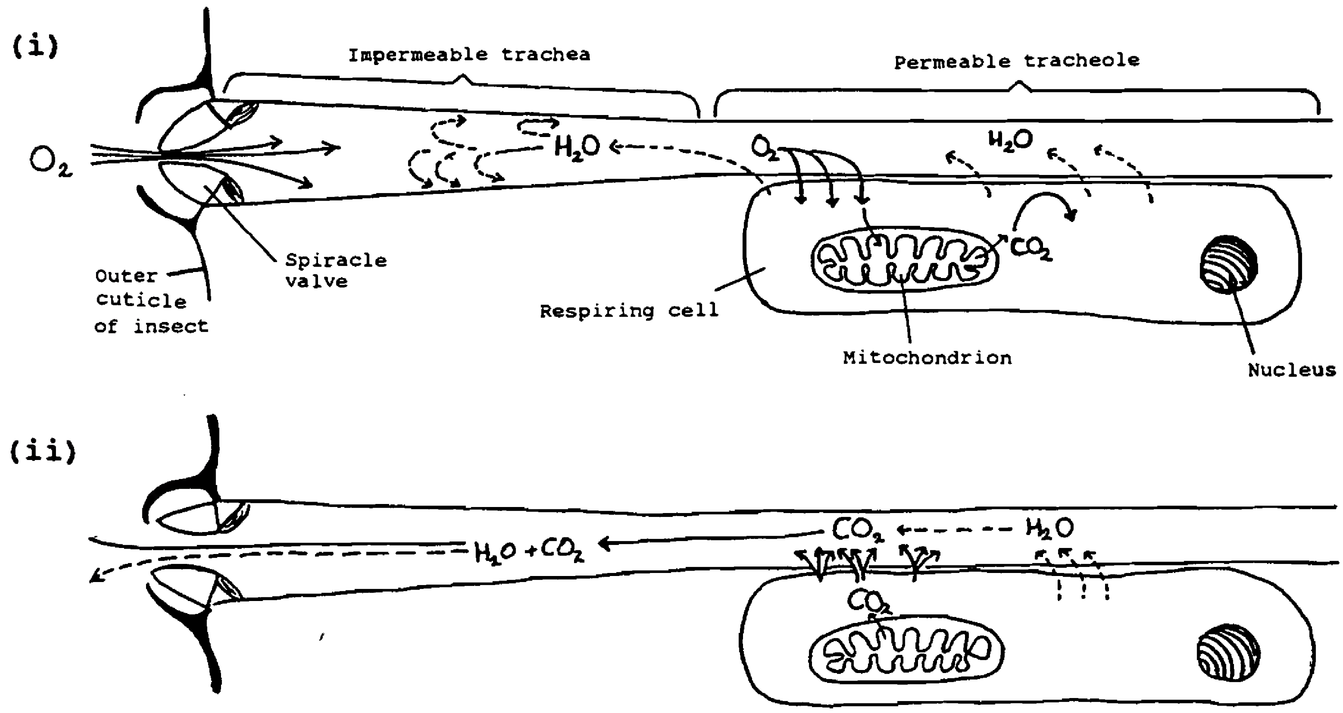

Figure 3 Gas exchange inside the tracheole.

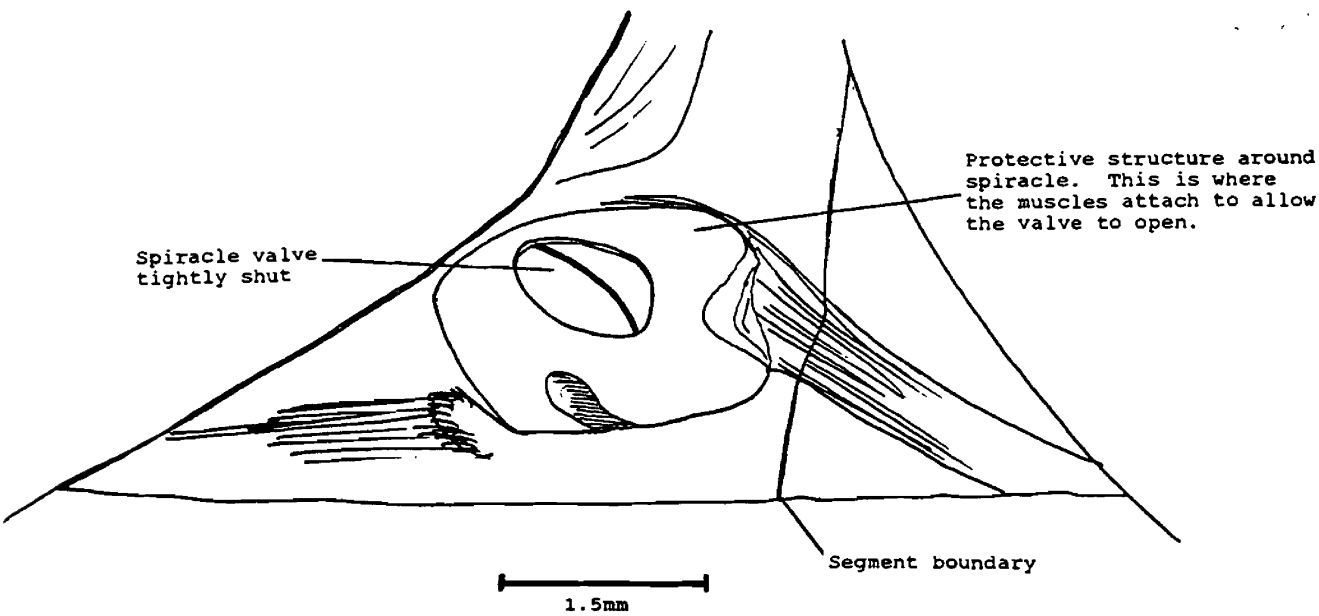

Figure 4 Closed spiracle on 4th abdominal segment of ♀ *Gigantophasma pallipes* Sharp

some of the larger insects eg. *Extatosoma tiaratum*, rhythmically opening and then almost closing, so controlling this process (see Figures 4 and 5). In a confined adult *Carausius morosus* I measured a rate of about three to four cycles per minute in a very humid environment, although I would expect the cycles to be much longer than this if the insect were short of water.

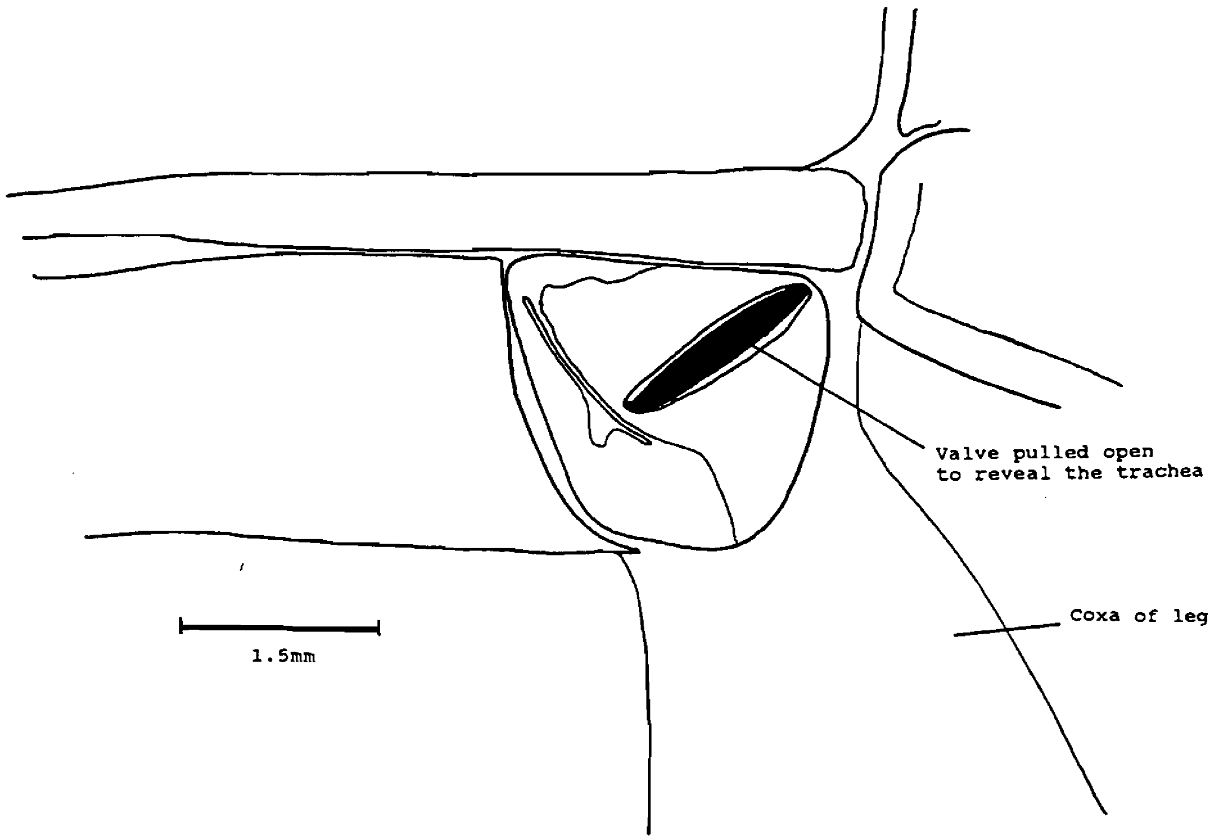

Figure 5 Open spiracle on 2nd thoracic segment of ♀ *G. pallipes*.

(3) Excretion

The excretory system is possibly the most specialised defence that insects possess against excessive water loss. All animals need a way to get rid of toxic waste products, and this is especially important for herbivores such as phasmids which may have to cope with a whole range of poisons that the plants on which they feed produce to ward them off. This is especially true for fern eaters such as *Carausius sechellensis* which may even have to deal with cyanide, which some ferns produce as a defence against herbivory.

Insects excrete substances from their haemolymph by using their malpighian tubules. These are a number (two to several thousand) of blind-ended tubes that splay out from the junction between mid and hind gut. The tube walls are only one cell thick, but still, in comparison with most other animals, are relatively impermeable to anything unless given specific hormonal cues to open up the various ion channels, or to activate pumping mechanisms. This impermeability creates a very slow flow rate into the tubes and consequently throughout the excretory system, and this in turn allows reabsorption of a lot of the water from the tubules and later from the hind gut. The basic mechanism for filtration is to pump potassium (K^+) ions out of the haemolymph of the insect and into the lumen of the tubule. This causes water to follow by osmosis, and then other solutes may follow, possibly including molecules of large molecular weight (e.g. up to 10000g/mole), which enter through small gaps between the cells. Added to this is the active transport of various specific toxins which particular species might encounter during their lives, such as alkaloids from plants, or magnesium and sulphate ions from salty water. This process is summarised in figure 6.

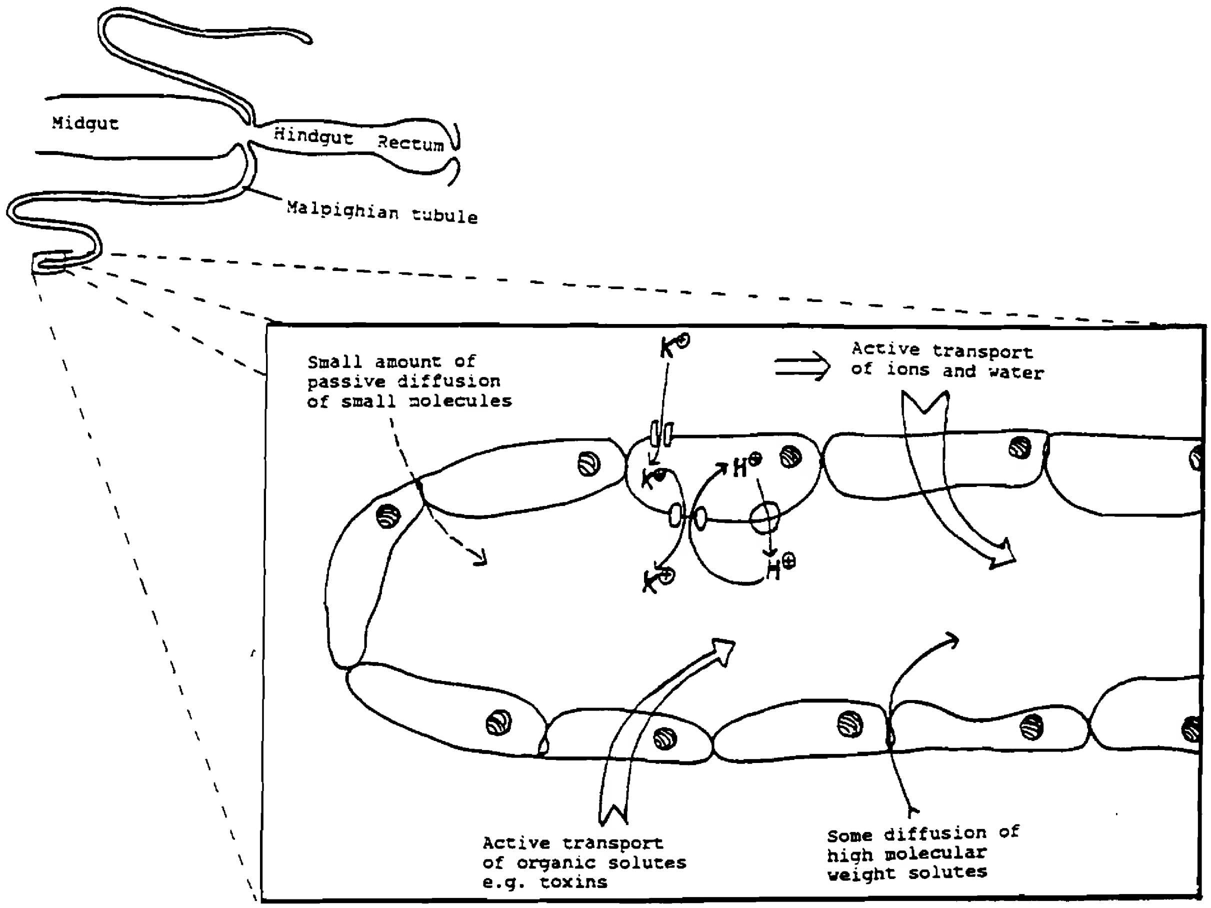

Figure 6 Excretion of substances in the malpighian tubules.

An important feature of insect water conservation is their "choice" of excretory product. Mammals excrete mostly urea, which requires around 50-100cm^3 of water for every gram of nitrogen excreted. Insects (along with many reptiles and birds) go one better and excrete uric acid. This is virtually insoluble, especially as potassium urate to which it is converted in the tubules of the insect, and consequently only needs 10cm^3 of water to excrete one gram of nitrogen. It is a little more energetically costly to make than urea, but the water-saving advantages seem to outweigh this slight disadvantage. Even this water may be absorbed again into the haemolymph in some insects to cut down even further on water losses. Nearly all of the useful solute and water reabsorption occurs in the hind gut of the insect. Amino acids and sugars are hauled back into the insect, probably via coupled transport to ions which the insect will be pumping back into its body, and this will draw some water out of the tubes back into the insect. However, the bulk of the water retrieval occurs in the rectum of the insect, again using just a single layer of cells to perform the entire concentrating task as is shown in figure 7. The effectiveness of this retrieval is shown in that many species of phasmid drop almost completely dry faeces. This concentration of wastes and toxins in the rectum could pose a serious danger to the living cells in the rectum lining. These are protected, however, by a lining of cuticle, impermeable to the large toxin molecules, but permeable to water and ions. This, therefore allows nearly all the water to be removed from the faeces without harm to the insect.

Combining these three approaches to the problem of water loss, the phasmids and other insects

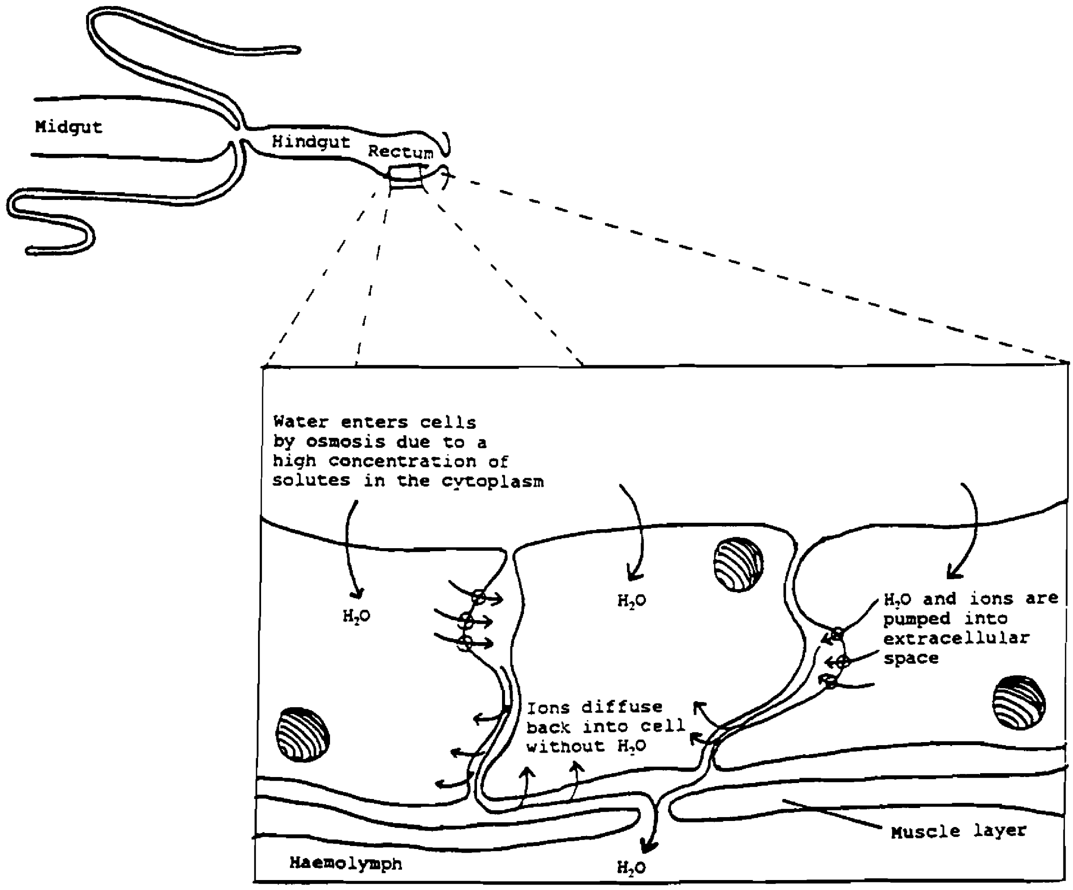

Figure 7 Reabsorption of water in the rectum.

have managed to survive in sizes and climates inaccessible to other terrestrial animals, and have thus been able to increase in number and diversity to the millions of species alive today. There is a final twist to the story, however, in that phasmids do not always want to be conserving water. A newly emerged imago of some holometabolous flying insect species, such as some Diptera, may lose up to 80% of their body water on their final moult to lighten themselves for flight (Maddrell, lecture 1995). Also, during flight, a lot of "metabolic water" is produced from the aerobic breakdown of glucose in the flight muscles. This amount can quickly exceed the amount being excreted by the malpighian tubules, and would therefore weigh the insect down. In both these cases, therefore, the excretory system is hormonally thrown into reverse, excreting phenomenal amounts of fluid very rapidly (for example, *Rhodnius* sp., a blood sucking bug from South America, can excrete its own body weight in water in only 15 seconds after a full blood meal), so quickly reducing the water load.

In conclusion, I hope that this summary of insect osmoregulation has shown how a few relatively simple structures (certainly in comparison with the mammalian kidney!) have enabled the phasmids and other insects to exercise exquisite control over their body water, allowing them to adapt to a huge range of environmental conditions.

Bibliography and Acknowledgements

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